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# THE OCCURRENCE OF B. TETANI IN SOIL AND ON VEGETABLES. VIII \*

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A number of enrichment cultures prepared in the course of the studies on the distribution of *B. botulinus* in the soil of the United States, Switzerland and China, produced on subcutaneous inoculation in guinea-pigs typical symptoms of tetanus. In every instance neutralization of the toxin was accomplished by the simultaneous injection of 1,000 units of commercial antitoxin. Unfiltered but centrifugalized cultures which caused tetanus were tested for *B. botulinus* toxin by feeding portions to guinea-pigs and mice. One sample of the soil from Maryland was found to contain the toxin of *B. botulinus*, type B, and that of *B. tetani*. The data, dealing with the distribution of the tetanus bacillus in 2,379 soil and vegetable samples collected in the United States, Europe and China, are the result of casual observations. No attempt has been made to study the factors necessary to demonstrate this bacillus in mass enrichment cultures. It is known that the toxicogenic capacity of *B. tetani* is greatly reduced in the presence of other aerobes and anaerobes, and it is quite possible that the conditions chosen for the study of *B. botulinus* were not always suitable for the production of a potent tetanus toxin. In order to stimulate a study of the distribution of *B. tetani* in nature, however, the few observations are briefly summarized in table 1.

TABLE 1  
DISTRIBUTION OF *B. TETANI* IN VARIOUS STATES

Arkansas.....	2 uncultivated virgin soil samples
California.....	Horse trail on Mt. Lowe, near Los Angeles
Indiana.....	1 pasture and 1 vegetable (swiss chard)
Maryland.....	1 uncultivated virgin soil sample, together with <i>B. botulinus</i> , type B
Michigan.....	2 garden soil specimens
Minnesota.....	2 garden soil specimens
New York.....	3 virgin soils collected around Mt. Baker, near Saranac Lake
Rhode Island.....	1 cultivated field (corn) and on 1 spinach specimen
West Virginia.....	1 virgin soil specimen from the Alleghany Mountains
Wisconsin.....	3 soil specimens from a heavily manured celery farm near Milwaukee
Switzerland.....	1 vegetable (brussels sprouts) from the vicinity of Bern
China.....	1 cultivated field near Pekin

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It will be noted from the data presented in this table that nine of the states of America which furnished enrichment cultures with *B. tetani* are topographically east of the Mississippi. No western soil material, with the exception of one (trail dust on Mt. Lowe, Calif.), produced cultures containing *B. tetani*. These differences are, on account of the large number of cultures involved, sufficiently striking to justify further discussion. Three hundred and ninety-seven specimens from 9 (Arkansas, Indiana, Maryland, Michigan, Minnesota, New Jersey, Rhode Island, West Virginia and Wisconsin) states mentioned in table 1 furnished 18 cultures, while 991 cultures prepared with similar samples from California, Oregon, Washington, Montana, Idaho, Nevada, and Wyoming gave only 1 culture containing *B. tetani*. During a study of 624 specimens which originated in California, special attention was paid to the occurrence of tetanus bacilli in mountain soil, but the negative results prompted the conclusion that the spores of *B. tetani* are rare in comparison to those of *B. botulinus*. As it is known that well-manured garden soil has yielded tetanus bacilli in the hands of various observers, it is rather surprising to find the numerous specimens of garden and manured field soil from Oregon, Washington and California free from this anaerobe. It has been suggested that these results are due to the technic employed, and in particular the method of testing the toxicity of the enrichment culture has been suspected as inadequate. In this connection it should be stated at once that the tetanus toxin in the enrichment cultures was always recognized in the course of the neutralization tests with *B. botulinus* anti-toxin. As a rule, all 3 guinea-pigs developed tetanus. The criticism that the presence of tetanus spores cannot always be demonstrated in mixed cultures is probably true for a small number of specimens, but this must apply equally to the samples collected in the East and Middle West, as the same method was employed throughout the entire series of studies. The evidence permits, therefore, only one conclusion: *B. tetani* is less prevalent in the soil of western states than in that of the eastern and middle western states. It is naturally possible that permanent infestation of the regions originally free from it may occur through commercial and industrial agencies, just as certain sections of California (Sacramento delta) have become permanently infected with anthrax spores from the offal and residues washed down into streams from tanneries, hair factories and improper burial places of animal cadavers.

The demonstration of tetanus spores in garden soil and on vegetables collected in eastern territories, Switzerland and China is quite in accord

with the well-known facts established many years ago by Nicolaier,<sup>1</sup> Bossano,<sup>2</sup> Bisserié,<sup>3</sup> Sanchez-Toledo and Veillon,<sup>4</sup> Rabinowitch,<sup>5</sup> and others. The association of tetanus spores with fertilization and manure has always been supported by an old theory derived from the French, which claims that the tetanus bacillus is a regular inhabitant of the intestines of the horse and other species of domestic animals. Even human feces have, according to Pizzini,<sup>6</sup> in Italy, and Ten Broeck,<sup>7</sup> in China, been found to harbor this anaerobe. According to the data presented in table 1, spores of *B. tetani* are found in virgin and uncultivated forest soil where plant material is undergoing fermentation and decay. It is, therefore, quite conceivable that this anaerobe can, just as *B. botulinus*, multiply in symbiotic relation with other anaerobes or aerobes, wherever protein material undergoes putrefaction. The presence of the tetanus bacillus in so many places outside the intestinal canals of animals suggests much more extensive breeding places than the animal body. Irrespective of the recent findings of Ninni,<sup>8</sup> who failed to find the spores of *B. tetani* in the soil from mountainous regions and elsewhere free from the dejecta of domestic animals, in studying *B. tetani* careful consideration should be given the facts established for *B. botulinus*. The entire problem of breeding places of all pathogenic anaerobes deserves renewed investigation in the light of the data presented in the papers dealing with the distribution of *B. botulinus* in nature.

#### CONCLUSIONS

The spores of *B. tetani* are frequently encountered in well manured, cultivated or garden soil, and on vegetables obtained from several states east of the Mississippi, Switzerland and China. In the United States even virgin forest soil has yielded cultures of *B. tetani*. The soil of the western states is relatively free from this anaerobe.

<sup>1</sup> Deutsch. med. Wchnschr., 1884, 10, p. 842; Beitr. z. Aetiologie des Wundstarr Krampfes, Göttingen, 1885.

<sup>2</sup> Rev. de méd., 1889, 9, p. 102.

<sup>3</sup> Thèse, Paris, 1894, No. 126.

<sup>4</sup> Semaine méd., 1890, 10, p. 45.

<sup>5</sup> Arch. f. Hygiene, 1907, 61, p. 103.

<sup>6</sup> Riv. d'igiene e san. pubbl., 1898, 10, p. 170.

<sup>7</sup> Personal communication, and Jour. Exper. Med., 1922, 36, p. 267.

<sup>8</sup> Ann. d'igiene, Rome, 1920, 30, p. 756.